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DEVELOPMENT OF A MINIMALLY INVASIVE BONE MARROW HARVEST DEVICE AND METHOD FOR THE RAPID EXTRACTION OF BONE MARROW FOR USE IN BONE MARROW TRANSPLANTATION AND STEM CELL THERAPY

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The bone marrow contains a rich supply of hematopoetic, mesenchymal and other stem cell populations. Bone marrow and peripheral blood–derived hematopoetic stem cells are currently used in > 40,000 bone marrow and peripheral blood stem cell transplants each year worldwide. Adult bone marrow–derived stem cells may soon be used in various stem cell–mediated regenenerative therapies, resulting in an increased need for a simplified, economical acquisition of bone marrow. Allogeneic marrow, or G-CSF mobilized marrow, may prove to be superior to PBSC for several transplantation indications, and thus improved harvest methods are needed.

Current methods of acquiring stem cells are tedious and expensive. Traditional methods for harvesting bone marrow from patients are crude and generally require 100 or more separate insertions of a large trocar needle into the donor's ileac crest. Serial trocar insertions allow aspiration of a small volume of bone marrow each time, eventually obtaining a volume of marrow containing an adequate number of stem cells. Marrow donors usually require general anesthesia, multiple staff, and often an overnight hospital stay. Peripheral blood stem cell harvests, alsthough less invasive, require the donor to undergo several days of expensive G-CSF administration, followed by many hours of apheresis. Traditional bone marrow harvests and peripheral blood transplants incur average charges approaching $15,000 per harvest.

We have developed a novel harvest device, the MarrowMiner, for rapid, minimally invasive extraction of bone marrow. This device requires only 1 or 2 separate bone marrow entry sites, because after entry into ileac crest, the device can access most of the accessible marrow space. We anticipate that the MarrowMiner device will enable a single operator to harvest bone marrow in a short period with only local anesthesia required in the outpatient setting, significantly facilitating convenient, on- demand stem cell collection while significantly reducing harvest costs. Collected marrow could be used immediately or undergo stem cell enrichment and manipulation for various current and potential future therapeudic indications. Results from the MarrowMiner device's preclinical development in miniature swine and human cadavers will be presented.

HEMATOPOIESIS/MESENCHYMAL CELLS

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IN VITRO UMBILICAL CORD BLOOD EXPANSION RESULTING IN UNIQUE CD34^{BRIGHT} CELL POPULATION THAT ENGRAFTS IN NOD/SCID MICE

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Umbilical cord blood (UCB) has gained recognition as a viable alternative to bone marrow or mobilized peripheral blood for hematopoietic stem cell transplantation and is being used for treatment of mainly pediatric patients with various malignant or genetic blood disorders. The limited number of cells contained in a single cord blood unit remains the major bottleneck for broader application of this treatment in adult patients. Extensive research is being focused on strategies for overcoming this bottleneck. Expansion of cells from a single cord blood unit or combining 2 cord blood units for a single transplantation are the 2 most common strategies currently in clinical evaluation.

Our research effort is focused on the expansion of umbilical cord blood cells. At BD Technologies, we have developed a unique higher-throughput technology platform that we have used successfully to identify a serum-free in vitro culture condition that supports expansion of UCB-derived cells. Expansion of UCB cells in this condition results in a unique cell population, characterized by a high level of glycoprotein CD34 expression. Characterization of this expanded population using flow cytometry, colony-forming assays, and gene array analysis indicate that this expanded cell population contains more primitive stem/progenitor cells than the remainder of the CD34+ population after expansion. When transplanted into sublethally irradiated NOD/SCID mice, these cells were found to engraft in the bone marrow and to develop CD19+ B-cell, CD33+ myeloid, CD34+ stem and progenitor, CD14+ monocyte, and CD42+ megakaryocyte lineages. Secondary transplantation studies indicate the long-term engraftment potential of the expanded cells.

In addition, when transplanted subcutaneously on porous polymer carriers into NOD/SCID mice, the expanded cord blood cells are found to initiate neovascularization within the carrier. Immunohistochemistry revealed that human cord blood cells were incorporated into the new vasculature. We conclude that our culture condition supports expansion of UCB cells that may have relevance for therapeutic applications in humans.

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BONE MARROW–DERIVED MESENCHYMAL CELLS DELAY ALLOGENEIC REJECTION

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Bone marrow–derived mesenchymal stem cells (BM-MSCs) have been demonstrated to have immunosuppressive effects in mice and humans. These findings suggest that they may be exploited in organ transplantation settings. We investigated immunoregulatory aspects of rat BM-MSCs. Bone marrow was extracted from 6- to 8-week-old DA rats and plated in 10% complete medium. Every 48 hours, nonadherent cells were discarded. The immunosuppressive effects of these cells in an allogeneic mixed lymphocyte reaction were investigated. MSCs dramatically suppressed MLR response (99%) when added at a concentration of 10^5 MSCs. Suppression was dose-dependent. In ConA cultures, MSC suppression of lymphoid cells was maximal at 10^5 cells. MSC suppression was not MHC-restricted, because Lewis-derived MSCs also suppressed MLR and ConA responses (> 90%). Immunoregulatory levels of IL-6, IL-10, and TNF-α were detected in supernatant of MSCs (BioPlex assay). The ability of MSCs to suppress allogeneic skin grafts was evaluated in an allogeneic rat model. Allogeneic skin grafts survived for 7 days without immunosuppressive therapy. In preliminary experiments, MSCs (derived from DA) were injected either as a single dose (10×10^6/kg) or as multiple doses given every 2 days (on days 0, 3, and 5). MSC injections, either a single injection or multiple injections, delayed skin graft rejection (days 8–12 or 14). Multiple MSC injections (10×10^6/kg) suppressed intestinal transplantation rejection in a rodent heterotopic intestinal transplantation model as well. The results suggest that MSCs may have clinical utility in transplantation settings.

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HUMAN MESENCHYMAL STEM CELLS AFFECT IGG PRODUCTION INDUCED BY LIPOPOLYSACCHARIDE, CYTOMEGALOVIRUS AND VARICELLA ZOSTER VIRUS IN HUMAN SPLEEN CELLS

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Human mesenchymal stem cells (MSCs) suppress T-cell proliferation and formation of cytotoxic T lymphocytes in vitro. In vivo, MSCs prolong skin allograft survival and mitigate severe graft-versus-host disease. The immunomodulatory properties of MSCs have focused on T cells. We have examined the effects of MSCs on human splenic B-cell IgG secretion. IgG response was analyzed using the ELI-spot assays, after stimulating of human spleen cells with lipopolysaccaride (LPS), cytomegalovirus (CMV), or varicella zoster virus (VZV) antigens with or without 10% irradiated MSCs. Unstimulated spleen cells gave 30 ± 9 Spot-forming units (SFUs)/

100,000 cells, and after stimulation with LPS, it was 403 ± 88 (n = 28, adjusted to 100%). The addition of 10% irradiated human MSCs gave 76 ± 9%. Despite this suppression of IgG secretion, we saw high variations in the experiments, as well as increased IgG secretion. Stimulation with CMV gave a median of 330 ± 98 SFU/100,000 cells (n = 3), and the addition of 10% MSCs to the culture reduced the response to 150 ± 63. Stimulating spleen cells with VZV gave 830 SFC/100,000 cells, which was reduced to 84 ± 7 with 10% MSCs present. Enriched B cells were negatively selected using immunomagnetic beads (Dynal, Norway). Using enriched B cells, IgG production stimulated by LPS were variably inhibited, unchanged, or stimulated after addition of 10% MSCs in the culture, giving an average of increased IgG production (170 ± 53% compared with 100% in the positive control; n = 5). Variations between different MSCs were similar to those using crude spleen cells, indicating that MSCs most likely have a direct effect on B cells, but because there might be some contaminating T cells remaining in the culture (flow cytometry showed < 0.5% T cells), we can not rule out that MSCs inhibit T cells and then in-direct the B cells. When T cells are activated in mixed lymphocyte cultures or by phytohemagglutinin, MSCs in high concentrations, 10% to 40% in the culture, mainly inhibit proliferation, whereas low concentrations, 1%, in some experiments enhance lymphocyte proliferation. For IgG secretion induced by LPS and viral antigens CMV or VZV, 10% MSCs in culture inhibits IgG secretion in most experiments, although a high variation in responses is seen with this concentration. To conclude, MSCs inhibited B-cell IgG secretion by human spleen cells induced by CMV and VZV. However, induction by LPS on crude spleen cells or purified B cells mainly stimulated IgG production.

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DURABLE ENGRAFTMENT OF AMD3100-MOBILIZED HEMATOPOIETIC STEM CELLS IN A CANINE AUTOLOGOUS AND ALLOGENEIC MODEL

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AMD3100 is a synthetic molecule that inhibits binding of stromal cell–derived factor-1 to its receptor, CXCR4, expressed on hematopoietic cells. AMD3100 treatment of animals or humans results in mobilization of hematopoietic progenitor cells. Peripheral blood mononuclear cells (PBMCs) mobilized with a combination of AMD3100 plus G-CSF stably reconstituted autologous hematopoiesis in cancer patients following myeloablative conditioning. However, the engraftment potential of AMD3100-PBMC used alone has remained unproven. We therefore studied this potential in the autologous and DLA-identical littermate canine hematopoietic cell transplantation (HCT) models. Pharmacokinetic studies performed in 4 dogs given a single dose of AMD3100 (4 mg/kg SC) demonstrated a 3- to 10-fold increase in CD34+ cells measured by flow cytometry, and a 2- to 5-fold increase in CFU-GM and BFU-E within 8–10 hours of AMD3100 administration. For autologous HCT, 4 dogs received a single dose each of AMD3100 (4 mg/kg) followed by 3–4 hours of leukaphereses initiated 6–7 hours after AMD3100 administration. Thereafter, dogs were given a single dose of myeloablative total body irradiation (TBI), 920 cGy at 7 cGy/min, followed by infusion of autologous AMD3100-PBMC (median CD34 cell count, 3.9×10^6/kg). Neutrophil and platelet engraftment occurred at medians of 9 days (range, 7–10 days) and 25 days (range, 23–38 days), respectively, after HCT. At a median follow-up of 11.5 months (range, 8–12 months), all 4 dogs had normal marrow function. Because a contribution of surviving endogenous stem cells to marrow function could not be ruled out in the autologous HCT model, we next evaluated the engraftment potential of AMD3100-PBMC in the allogeneic HCT model. Four dogs were given AMD3100-PBMC (median CD34 cell count, 3.4×10^6/kg) from DLA-identical littermates after conditioning with 920 cGy TBI. Postgrafting cyclosporine (5 mg/kg twice daily) was given from days −1 to +35. Neutrophil and platelet engraftment occurred at medians of 9 days (range, 8–10 days) and 26 days (range, 26–37 days), respectively. At a median follow-up for living recipients of 162 days (range, 72–178 days), 3 dogs were alive and demonstrated 99%-100% donor chimerism by VNTR analysis. One dog had early evidence of engraftment but was euthanized 18 days after HCT because of canine HSV infection and pancreatitis. In summary, both autologous and allogeneic AMD3100-PBMC transplants were associated with timely and durable engraftment in dogs conditioned with myeloablative TBI.

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ALLOGENEIC T CELLS INDUCE CORD BLOOD CD34+ OR CD133+ CELLS RAPID PROLIFERATION AND DIFFERENTIATION INTO DENDRITIC CELL PRECURSORS

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In this study we tested whether initial contact with allogeneic lymphocytes may increase human cord blood (CB) hemopoietic stem cells (HSCs) alloantigen-presenting capacity. Irradiated immunomagnetically purified CB CD34+ and CD133+ HSCs induced similar proliferation (mean stimulation index, 19 ± 5 and 16 ± 5, respectively) of allo-responders in primary mixed leukocyte culture (MLC). Cocultures of nonirradiated CD34+ or CD133+ cells with irradiated allogeneic T cells for 6 days resulted in a brisk proliferation of HSC fractions (mean stimulation index, 17 ± 2). HSC proliferation was inhibited by B7:CD28 blockade with CTLA-Ig. HSC that had been cocultured with irradiated allogeneic T cells for 6 days, were then rechallenged as stimulators in MLC, and induced a significantly greater T-cell alloresponse as compared with the same freshly isolated HSCs (stimulation index, 134 ± 54 vs 24 ± 8) ($P = .03$). The phenotypic kinetics of CD34+ and CD133+ CB cells on contact with irradiated T cells showed a rapid up-regulation of CD86 within 24 hours (on average from 2% ± 1% to 7% ± 2%, and from < 1% to 4% ± 1%, respectively). At 3 and 6 days of culture, both CD34+ and CD133+ CB cells became larger and included a population of HLA−DR+ CD14+ CD86+ CD11c+ progenitors, on average 16%, consistent with the phenotype of DC precursors. Also, irradiated allogeneic lymphocytes induced the generation of 8% CD1a+ DCs after 8 days of culture with HSCs. To assess the effect of allogeneic lymphocytes on CB CD34+ cell-derived DC generation in vivo, 10 NOD/SCID mice were irradiated (300 cGy) and injected with 0.20×10^6 CD34+ CB cells and autologous (CD34/auto) or allogeneic (CD34/allo) MNC (ratio 1:2), or with autologous or allogeneic cells alone as control. At 6 weeks after transplant, marrow cells were harvested, and human CD45+ cells were on average 1.8% in CD34/auto and 3.2% in CD34/allo. Although similar proportions of B cells (CD45+CD19+) and monocytes (CD45+CD14+) were detected in the two groups, a greater number of myeloid dendritic cells (CD45+BDCA−1+) was observed in CD34/allo than in CD34/auto (0.45% ± 0.30% vs 0.04% ± 0.04%). These findings suggest that allogeneic lymphocytes may induce a rapid differentiation of HSC into DC with potent alloantigen-presenting capacity. Future studies aimed at either selectively abrogating this subset of progenitors or blocking HSC:T-cell reciprocal activation will be developed in the attempt of facilitating the induction of tolerance in allogeneic HSC transplantation.

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SUFFICIENT NUMBERS OF CORD BLOOD DERIVED MESENHYMAL STEM CELLS FOR TRANSPLANTATION ARE GENERATED BY PLASTIC ADHESION OR DEPLETING METHODS

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Cord blood (CB) contains nonhematopoietic stem cells referred to as mesenhymal stem cells (MSCs). These cells contained in CB can be considered as "very young," and thus CB may be an excel-